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Variant Classification Assertion Criteria

Variant interpretation at Variantyx is a multi-step process involving literature review, technical genetic sequence analysis, review of case data, analysis of population frequency, computational predictions, and evidence-based classification. Small sequence changes (SSCs) in nuclear genes are classified based on the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) scoring guidelines (PMID: 25741868) and refinements published by the Clinical Genomics Resource (ClinGen) (e.g., PMIDs: 30192042, 31892348). Mitochondrial variant classification is based on specifications of ACMG/AMP scoring guidelines (PMID: 32906214). Classification of copy number variations (CNVs) is based on the standards published by ClinGen and ACMG (PMID: 31690835), with refinements by Variantyx cytogeneticists based on extensive internal case experience. Variantyx software also identifies short tandem repeat (STR) expansions from whole genome sequencing data. Since there are no published variant classification guidelines for STRs, Variantyx classifies expansions based on gene-specific, peer-reviewed literature and population databases for normal, intermediate, and expanded allele thresholds, and applies data on relevant sequence interruptions to determine pathogenicity. Interpretations at Variantyx are performed by a team of skilled variant scientists and undergo multiple levels of review by clinical and molecular laboratory directors.

Nuclear SSCs, mitochondrial variants, and CNVs may be classified into one of five categories based on Variantyx standard operating procedures: pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign (LB), and benign (B).

The rules for combining ACMG lines of evidence for nuclear SSCs and mitochondrial variants are listed below. Variants with conflicting evidence or insufficient evidence to meet criteria for P, LP, B, or LB will be classified as VUS. Variantyx may increase or decrease the strength of the ACMG/AMP criteria based on the amount and quality of evidence. Nuclear SSCs and mitochondrial variants are scored using stand alone, very strong, strong, moderate, and supporting lines of evidence and can be combined using the following rubric:

Table of Evidence Combinations and Variant Classifications for Nuclear and Mitochondrial SSCs

Evidence Combinations	Classifications				
	Pathogenic	**Likely Pathogenic**	**Variant of Uncertain Sig.**	**Likely Benign**	**Benign**
Combination 1	1 Very Strong AND ≥1 Strong	1 Very Strong AND 1 Moderate	Variants with a combination of conflicting evidence or insufficient evidence to meet criteria for other classifications.	≥2 Supporting	1 Stand Alone
Combination 2	1 Very Strong AND ≥2 Moderate	1 Strong AND ≥ 1 Moderate		1 Strong AND 1 Supporting	≥2 Strong
Combination 3	1 Very Strong AND 1 Moderate and 1 Supporting	1 Strong AND ≥2 Supporting			
Combination 4	1 Very Strong AND ≥2 Supporting	≥3 Moderate			
Combination 5	≥2 Strong	2 Moderate AND ≥2 Supporting			
Combination 6	1 Strong AND ≥3 Moderate	1 Moderate AND ≥4 Supporting			
Combination 7	1 Strong AND 2 Moderate AND ≥2 Supporting	PVS1 (Null variant in a gene with know LOF mechanism) AND PM2_Supporting (Sufficiently rare in control populations – inheritance and penetrance specific)			
Combination 8	1 Strong AND 1 Moderate AND ≥4 Supporting				

The rules for classifying CNVs are based on the ClinGen CNV Interpretation Calculator. The total scores are calculated by weighing the lines of evidence to determine a final score. The breakdown is as follows:

1. Pathogenic: Score 0.99 points or higher
2. Likely Pathogenic: Score between 0.90 and 0.98 points
3. VUS: Score between -0.89 and 0.89
4. Likely Benign: Score between −0.90 and −0.98 points
5. Benign: Score −0.99 or less

Pathogenic Variants:
These variants are considered disease causing either alone or in the presence of other pathogenic variants (e.g. *in trans* with a pathogenic variant to cause an autosomal recessive condition). Evidence supporting a pathogenic classification includes, but is not limited to:

a. A sufficiently rare (PM2_Supporting) missense variant in a nuclear gene found in *trans* with pathogenic variants in at least 10 affected, unrelated probands (PM3_Very Strong), and multiple lines of computational evidence support a deleterious effect on the gene or gene product (PP3).
b. A copy number loss which completely overlaps established haploinsufficient (HI) genes/genomic regions (1 full point).
c. A sufficiently rare (PM2_Supporting) *de novo* (PS2) mitochondrial variant for which cybrid studies show reduced function (PS3_Supporting), and the variant produces the same amino acid change as previously established pathogenic variants (PS1).

Likely Pathogenic Variants:
These variants are considered likely to be disease causing; however, the available evidence is not sufficient for a pathogenic classification. Evidence supporting a likely pathogenic classification includes, but is not limited to:

a. A sufficiently rare (PM2_Supporting) nonsense or frameshift variant in a nuclear gene where HI or loss of function (LOF) is a known mechanism of disease (PVS1).
b. A *de novo* copy number gain (0.45) where one breakpoint is within an established HI gene and the proband's phenotype is highly specific and consistent with what is expected for LOF in that gene (0.45).
c. A sufficiently rare (PM2_Supporting) in-frame insertion (PM4) into a protein coding mitochondrial gene which has been shown to segregate with disease in at least 5 maternal family members (PP1_Moderate), and multiple lines of computational evidence support a deleterious effect (PP3).

Variants of Uncertain Significance:
The available evidence is currently insufficient to determine the role of these variants in disease. Evidence supporting an uncertain classification includes but is not limited to:

a. SSCs with conflicting evidence.
b. CNVs which contain 0-24 protein coding genes and do not overlap known HI or triplosensensitive genes/genomic regions.
c. Variants in mitochondrial tRNA genes without cybrid/single fiber studies or known segregation with disease.

Likely Benign Variants:
These variants have suggestive evidence of benign effects. Evidence supporting a likely benign classification includes but is not limited to:

a. A synonymous variant in a nuclear gene not predicted to impact splicing (BP7) with greater than 1% frequency in healthy populations (BS1).
b. A copy number gain that does not contain protein-coding or other known functionally important elements (-0.60) inherited from an unaffected parent (-0.30).
c. A mitochondrial variant seen at higher heteroplasmy levels in healthy adult family members (BS2), and multiple lines of computational evidence suggest no impact on gene/gene product (BP4).

Benign Variants:

These variants are not considered to be causative for disease. Evidence supporting a benign classification includes, but is not limited to:

a. A variants in a nuclear gene where multiple functional studies demonstrate no significant difference between the function of mutant and wild-type proteins (BS3), and there are greater than 3 presumably unaffected heterozygous individuals in control databases in genes associated with early onset, highly penetrant, autosomal dominant disease (BS2).
b. A copy number loss completely contained within an established benign CNV region (-1 full point).
c. A mitochondrial variant found in healthy populations at >1% frequency (BA1).

References:

Richards et al. (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17 (5):405-24 (PMID: 25741868)

Abou et al. (2018) Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. Hum Mutat 39 (11):1517-1524 (PMID: 30192042)

Brnich SE, et al. (2019) Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. Genome Med 12(1):3 (PMID: 31892348)

McCormick EM, et al. (2020) Specifications of the ACMG/AMP standards and guidelines for mitochondrial DNA variant interpretation. *Hum Mutat* 2020;41(12):2028-2057. (PMID: 32906214).

Riggs ER et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med 2020 02 22(2):245-257 (PMID: 31690835)